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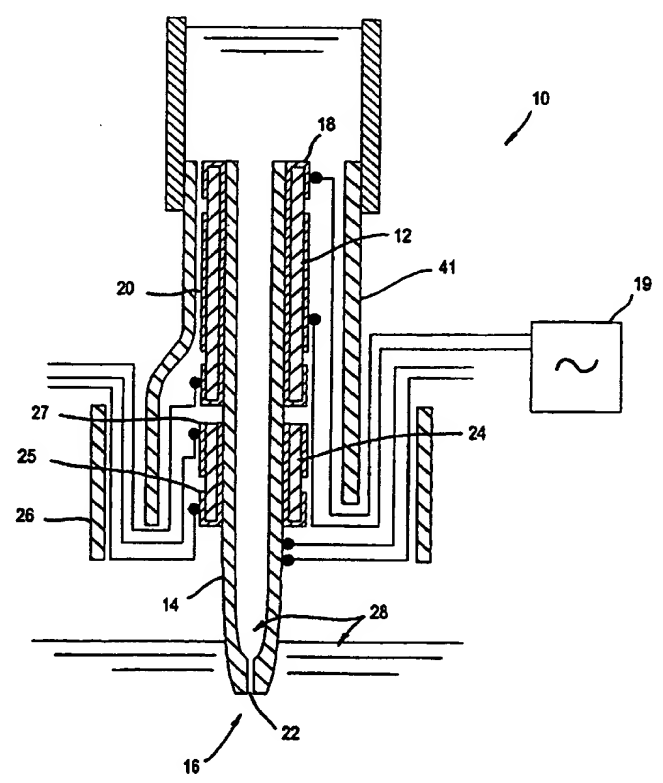
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(54) Title: PIEZOELECTRIC PIPETTING DEVICE

(57) Abstract

A piezoelectric actuated device for acquiring and dispensing fluid samples is described. Fluid samples are acquired or drawn up into the device by dipping the tip into a fluid and applying an alternating current electrical signal to the piezo-electric element. Removing the tip from the fluid and applying another electrical signal causes drops of fluid to be ejected for non-contact dispensing. The device optionally incorporates a second piezoelectric element functioning as a sensor to detect if it is empty, plugged or working properly. An optional heater, temperature sensor and feedback circuitry regulate the temperature of the device.



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PIEZOELECTRIC PIPETTING DEVICE

BACKGROUND OF THE INVENTION

The present invention relates to a piezoelectric actuated device for manually
5 or automatically transferring very small volumes of fluid. Transferring fluids from one
container to another is one of the most common tasks performed in a typical
chemical or biological laboratory. For example, various chemicals from different
containers may have to be mixed together and then the mixture may have to be
divided out equally or 'aliquoted' into other containers or onto microscope slides or
10 some other substrates. Currently transferring of fluids is done either by hand using
manual pipettors or automatically using an automated robotic pipetting instruments.
Motivated by the high costs of chemical reagents there is a trend in chemical and
biological laboratories to perform experiments using smaller and smaller fluid
samples.

15 Prior to the 1970's fluid transfer was done in laboratories by hand using glass
tubes called pipettes. Samples were aspirated by sucking on the end of the pipette
by mouth like a straw and then sealing the end with a finger. Fluid samples were
then dispensed or aliquoted by briefly unsealing and then resealing the pipette with
the finger allowing a small volume of fluid to flow out. This method required a fair
20 amount of dexterity and had some serious drawbacks in terms of ergonomics,
precision and health risks. During the same time period glass capillaries were also
used for transferring smaller volumes of fluid with greater precision. These
techniques for manually transferring fluid samples were made obsolete in the early
1970's by the introduction of the manual pipette as described, for example, in U.S.
25 Patent Nos. 3,613,952, 3,766,785, 3,827,305 and 3,991,617, with ejectable,
disposable plastic tips. Currently the manual pipette is a ubiquitous and
indispensable tool for transferring fluid samples in the modern laboratory.

Although manual pipetting is a big improvement over earlier techniques it is
tedious for the human operator and can cause repetitive motion injuries as well as
30 being prone to human error. Currently, a great variety of automated pipetting
instruments are commercially available which address these specific drawbacks.

These instruments, as advanced by Tecan AG of Hombrechtikon Switzerland, typically use a Cartesian X,Y,Z robot to move a pipetting head among various aspirating, dispensing and washing stations. The various types of instruments currently available differ mainly in the mechanisms used to acquire and then

5 dispense fluid samples. The most common mechanism for fluid transfer is the syringe pump as used in instruments manufactured by Tecan, Hamilton Company, Cavo Scientific Instruments, Robbins Scientific, Qiagen and Tomtec among others. In most cases the syringe pumps and pipette tips are connected via long flexible tubing, however, in the instruments supplied by Tomtec and Robbins Scientific the

10 syringe pumps are mounted directly to the back of the pipette tips. The smallest volume that can be accurately transferred using syringe pumps is approximately 0.1 microliters. U.S. Patent. Nos. 5,743,960 and 5,741,554 describe an instrument which combines a syringe pump with a solenoid valve allowing drops as small as 0.1

15 microliters to be ejected onto a substrate for non-contact printing or arraying applications. By contrast, the drops that are dispensed using the present invention are a thousand times smaller.

Another method for transferring small volumes of fluid for arraying applications is the use of pins as described in U.S. Patent No. 5,807,522. Instruments using pins for fluid transfer are used by Synteni, among other

20 companies, to generate DNA arrays and are commercially available, for example, from BioRobotics and GeneMachines. Using pins is a simple, robust and practical means for fluid transfer but it suffers from some limitations. First of all it is slow. The pins have to stop at each spot and then wait for over a second for capillary action to transfer the fluid onto the substrate. By contrast, the piezoelectric based dispensing

25 of the present invention is almost a thousand times faster. Pin based fluid transfer is sensitive to the wetting properties of the substrate. Also it can damage some substrates like Nylon membranes for example. These are not concerns for the piezoelectric dispensing which is non-contact. Pins generate relatively low density, poor quality arrays with approximately 50% variability in spot size. By contrast,

30 piezoelectric dispensers generate arrays with almost an order of magnitude higher density and better than 3% spot size variability. Finally, pins are limited to acquiring

and dispensing a fixed volume of fluid. Piezoelectric dispensers have thousands of times higher dynamic range. Sub nanoliter to tens of microliter volumes can be aspirated and subsequently dispensed in volumes ranging from 100 picoliters to several microliters per second.

5 A number of companies including Microdrop, Packard Instruments and GeSiM, use piezoelectric fluid dispensing devices to dispense drops of fluid with volumes on the order of 100 picoliters. Originally piezoelectric dispensing technology was used for "drop-on-demand" "ink-jet" printing. These devices as described in U.S. Patent No. 2,512,743 have a fluid filled chamber with an inlet at
10 one end and a nozzle on the other. A piezoelectric element induces an acoustic wave in the fluid causing a drop to be ejected from the nozzle. For printing applications ink is supplied to the back end of the piezoelectric fluid dispenser from a reservoir. For fluid transfer applications fluid is drawn up through the nozzle. The instruments supplied by Microdrop, Packard Instruments and GeSiM all use syringe
15 pumps to aspirate fluids up through the nozzle of the piezoelectric devices. Syringe pumps impose several serious limitations on current piezoelectric based pipetting instruments. It turns out that properly aspirating samples prior to dispensing is one of the most critical considerations for reliable operation of piezoelectric fluid dispensers. Syringe pumps can sometimes aspirate air bubbles and small particles
20 that can adversely affect the dispensing characteristics. Also, variations in back pressure in the flexible tubing leading to the syringe pumps causes the drop-on-demand dispensing properties to change. Packard Instruments uses a closed loop pressure controller to actively regulate this back pressure. This system adds to the cost and complexity of the instrument. Additionally the syringe pumps themselves
25 add significantly to the cost, size and complexity of the instrument and they limit the smallest fluid volumes that can be transferred to around 0.5 microliter. By contrast, with the present invention, volumes over 100 times smaller can be aspirated. Most importantly, aspirating is much more uniform and precise making dispensing more reliable and repeatable. In the present invention, the dispensers are opened to
30 ambient pressure so the back pressure always remains uniform. Additionally the novel sensing technology of the present invention detects almost instantly if there is

a problem with the dispensers, e.g. if they are clogged, empty or attempting to aspirate from an empty well.

The present invention provides improvements to the cylindrical piezoelectric fluid dispenser described in U.S. Patent No. 3,840,758. These improvements
5 enable bi-directional operation of the device, sensing of the operational state and thermal control. As a summary of some related devices. Humberstone in U.S. Pat. No. 5,518,179 describes a bi-directional device with a piezoelectric driven, thin, perforated membrane capable of drawing a fluid up through the perforations as well as dispensing fluid out through them. Beckman et al. in U.S. Pat. No. 4,432,699
10 describes a peristaltic piezoelectric pump with an internal load sensor. Hayes in U.S. Pat. No. 5,622,897 describes a process of manufacturing a drop-on-demand ink-jet print head having n-type and p-type thermoelectric carriers.

SUMMARY OF THE INVENTION

15 The invention provides a device enabling the automatic or manual transfer of small volumes of fluid from one container to another or from one container to the surface of a substrate. The device is a self contained, bi-directional pump in the sense that it can acquire fluid samples as well as dispense them. Acquiring samples in accordance with the present invention requires dipping the tip of the device into
20 the sample fluid. Dispensing, however, does not require any contact with either a fluid or solid surface. Fluid can be dispensed one drop at a time in a "drop-on-demand" mode or continuously at frequencies from over several thousand to fifty thousand drops per second.

In accordance with further aspects of the present invention, an integral
25 monitoring sensor detects if the piezoelectric pipette is functioning normally, is empty or if the nozzle is plugged. This type of feedback of the operational state of the device is critical for automated pipetting instruments to alert a human operator if the device requires some attention or to initiate an automated failure recovery procedure.

30 The characteristics of drop-on-demand ink-jet dispensers can change with temperature. In accordance with a further aspect of the present invention, a

piezoelectric pipette incorporates a built in temperature regulator. This helps maintain uniform dispensing characteristics even if the ambient temperature changes. The temperature regulator can also be used to raise the temperature of the fluid being dispensed. Elevated temperatures can reduce the viscosity of fluids that are too viscous to dispense at ambient temperature.

In summary, the invention is a compact, bi-directional pump for acquiring and dispensing small volumes of fluid with a built in monitoring sensor and temperature control. The invention is suitable for automated or manual fluid transfer applications.

Accordingly, the invention provides a diffuser valve pump including a pipette having a capillary having a first end including a nozzle and a piezoelectric actuating element adjacent to the capillary and an alternating current voltage source coupled across the piezoelectric actuating element. With the nozzle immersed in a sample fluid and the capillary at least partially filled with a working fluid, alternating current voltage applied to the actuating element causes sample fluid to be drawn into the capillary.

The invention further provides a pipette sensing arrangement for sensing a working condition of a pipette having a capillary with an end including a nozzle, a piezoelectric actuating element adjacent to the capillary, and first electrodes for applying electrical energy across the piezoelectric actuating element. The sensing arrangement includes a piezoelectric sensing element adjacent to the capillary and second electrodes for coupling the piezoelectric sensing element to a voltage sensor.

The invention still further provides a temperature control arrangement for a pipette having a capillary having an end including a nozzle, a piezoelectric actuating element adjacent to the capillary, and actuating electrodes for applying electrical energy across the piezoelectric actuating element. The temperature control arrangement includes a current source for passing an electrical current through one of the actuating electrodes, and a temperature sensor closely adjacent the capillary for sensing the temperature of the capillary.

BRIEF DESCRIPTION OF THE DRAWINGS

The features of the present invention which are believed to be novel are set forth with particularity in the appended claims. The invention, together with further objects and advantages thereof may best be understood by making reference to the following description taken in conjunction with the accompanying drawings, in the several figures of which like reference numerals identify identical elements, and wherein:

Figure 1 is a cross-sectional view of piezoelectric pipette embodying the present invention including a sensor, temperature controller, Teflon housing and reservoir;

Figure 2 is a cross-sectional view of piezoelectric pipette in a diffuser valve pump mode in accordance with the present invention with a reservoir attached to the back end and a mounting bracket arm for automated dipping into the sample fluid;

Figure 3 is a manually operated piezoelectric pipette;

Figure 4 is a cross-sectional view of piezoelectric pipette embodying further aspects of the present invention with a tube attached to the back end and another tube attached to the front end;

Figure 5 is a cross-sectional view of two piezoelectric pipettes back-to-back forming a reversible continuous flow pump in accordance with the present invention;

Figure 6 is a cross-sectional view of piezoelectric pipette with a separate sensor embodying the present invention;

Figure 7 is a graph of the power spectral densities of the sensors for water vs. Fluorinert fluids with the device operating in drop-on-demand mode illustrating a further aspect of the present invention;

Figure 8 is a block diagram of a dispenser actuation and sensor detection system embodying the present invention;

Figure 9 is a flow diagram illustrating an actuation and detection method embodying the present invention;

Figure 10 is a cross-sectional view of a piezoelectric pipette with a thermocouple and heating system including an inner electrode functioning as an integrated heating element embodying the present invention; and

Figure 11 is a block diagram of a temperature control system which may be employed with the pipette of Figure 10 in accordance with the present invention.

DETAILED DESCRIPTION

5 Referring now to Figure 1, it illustrates a piezoelectric pipette 10 with sensor, temperature control, housing and reservoir embodying the present invention. It includes an actuating piezoelectric ceramic tube 12 with a glass capillary 14 bonded concentrically on the inside. The glass capillary has a nozzle 16 formed on one end by heating and then polishing the end. Applying a voltage to the piezoelectric crystal
10 12 across an inner electrode 18 and an outer electrode 20 causes it to deflect which induces an acoustic wave in the fluid filled glass capillary 14. A drop is ejected from the nozzle if the acoustic energy is high enough to overcome surface tension at the nozzle opening 22. In accordance with the present invention, a separate sensing piezoelectric ceramic tube 24 is concentric with the glass capillary 14 and adjacent
15 to the actuating piezoelectric tube 12. These elements may also be seen in Figure 6 with the corresponding elements having corresponding reference numerals with an "a" subscript. The piezoelectric pipette is housed in Teflon heat shrink tubing 26 and 41 as shown in Figure 1. This housing is compact, provides mechanical protection and electrical insulation for the piezoelectric crystal.

20

Diffuser Valve Pump Mode

With the nozzle immersed in fluid 28 as shown in Figure 1 the device 10 operates as a diffuser valve pump capable of acquiring or drawing fluid samples up through the nozzle. Due to the shape of the nozzle, fluid has less resistance flowing
25 from the sample fluid up into the glass capillary than out. Periodically deflecting the piezoelectric crystal by applying an alternating voltage from an alternating current source 19 to the electrodes 18 and 20 causes fluid to flow in and out of the nozzle with a net inward flow due to the lower flow resistance in this direction. Based on the diffuser valve pump principle it is possible to draw up fluid samples into the
30 piezoelectric pipette and then to dispense them as a drop-on-demand 'ink-jet' printer.

The diffuser valve pump works over a large range of periodic excitation voltage frequencies, amplitudes and waveforms. Pumping speed increases with frequency and amplitude. This diffuser valve pump mode has been demonstrated to successfully pump fluid with excitation signal frequencies ranging from 6 KHz to 25
5 KHz and amplitudes from 20 V to over 90 V. Pumping was demonstrated with a variety of waveform shapes including saw-tooth, square-wave and sine-wave waveforms.

The glass capillary of the device must be at least partially filled with fluid for both drop-on-demand and diffuser valve pump modes to function properly. This
10 'working' fluid does not necessarily have to be the same as the sample fluid as illustrated in Figure 2 which shows another pipette 10b wherein corresponding elements are designated by corresponding reference numerals with a "b" subscript. Here the working fluid 30 and the sample fluid 28b are different. The working and sample fluids may be immiscible with each other allowing small fluid samples to be
15 drawn up without diffusing the sample into the working fluid. A wide variety of fluids immiscible in, for example, water in practicing the present invention may be selected including Fluorinert manufactured by 3M, 200 Fluid manufactured by Dow Corning and paraffin. Fluorinert has a good combination of properties for many applications including low viscosity, good bio-compatibility and it does not leave a residue when
20 dried out.

As also illustrated in Figure 2, the piezoelectric pipette 10b can be mounted on a supporting mounting bracket arm 32. This arm can be attached to an automated robotic instrument which dips the device into the sample fluid 28b for aspirating and then moves it to another station for dispensing. Alternatively, the
25 piezoelectric pipette 10b with wires 33 can be attached to a manual pipettor 34 having a disposable tip 36, as shown in Figure 3. In this case the device is dipped into the sample fluid by a human operator and then manually moved to another container or substrate for dispensing. The plunger 38 of the manual pipettor can be used for priming, rinsing and emptying the device.

30 Three different versions of the piezoelectric pipette 10b operating as a diffuser valve pump are shown in Figure 2, Figure 4 and Figure 5. As seen in

Figure 2, attaching a reservoir 40 to the back end 42 of the piezoelectric pipette 10b allows larger volumes of fluid 28b to be drawn up. As seen in Figure 4, by attaching tubing 44 and 46 to respective ends 48 and 42b respectively of pipette 10b, the piezoelectric pipette operates as a pump for a continuous flow system. A reversible continuous flow pump can be made by putting two piezoelectric pipettes 10b back to back as shown in Figure 5. In each of the embodiments of Figures 2 and 4, the voltage source 19 of Figure 1 may be used to apply an alternating current voltage across the inner and outer electrodes of the actuating piezoelectric elements. In the embodiment of Figure 5, separate voltage sources may be used for the actuating electrodes or a single source may be selectively applied to one or the other of the actuating elements.

Integrated Sensor

The devices 10 and 10a shown in Figure 1 and Figure 6 respectively each incorporate a separate piezoelectric element 24 and 24a respectively which functions as a sensor to monitor the operational state of the device. The sensing elements 24 and 24a are proximal to their corresponding actuating elements 12 and 12a respectively with respect to the nozzles 22 and 22a. The sensor 24, 24a detects vibrations induced in the fluid 28, 28a, the glass capillary 14, 14a and the actuating piezoelectric crystal 12, 12a. By analyzing the voltage signal generated by this sensor across electrodes 25 and 27 and 25a and 27a it is possible to identify the operational state of the device, specifically, if the dispenser is operating properly both in drop-on-demand and diffuser valve pump modes of operation. Specifically it is possible to detect if the device is working properly, if it is empty or full when aspirating or dispensing, if the nozzle is partially or completely plugged when aspirating or dispensing, what type of fluid is being dispensed or if the device is trying to aspirate fluid from an empty container. The power spectral densities of the time signals are computed to make it easier to distinguish the differences between these various operating conditions. For example, Figure 7 shows the power spectral density (PSD) of the output from the sensor while the dispenser is dispensing in drop-on-demand mode. The PSDs in Figure 7 were computed by

averaging the fast Fourier transforms (FFTs) of 16 individual Hanning windowed data records of 128 points each sampled at a frequency of 375 KHz. The figure shows the difference in the sensor output if water 43 vs. Fluorinert 45 is being dispensed. At 90 KHz there is a difference of three orders of magnitude between the PSDs of water 43 and Fluorinert 45. Detection software will compare the PSDs at this frequency to determine if the sample fluid (water) or the working (Fluorinert) fluid is being dispensed.

A block diagram of the dispenser actuation and sensor detection system 50 is depicted in Figure 8. It incorporates a digital computer 52, drive electronics 54 for the actuating piezoelectric element 12, 12a, the piezoelectric pipette 10, 10a itself with an integrated sensor 24, 24a and an analog to digital converter (ADC) 56 for the sensor signal. The computer 52, upon implementing operating instructions in accordance with this embodiment of the present invention forms an actuation and detection stage 58. A flow diagram 60 of the actuation and detection stage operation is depicted in Figure 9. First in step 62, an actuating trigger 51 command to actuate the device is issued to the drive electronics 54. The drive electronics 54 in turn generates an actuating voltage 53 to drive the actuating piezoelectric element 12, 12a with a voltage waveform. To obtain the sensor data, for Figure 7 for example, the piezoelectric element may be driven with a 60 microsecond, 35 volt amplitude pulse. The voltage causes the actuating piezoelectric element to deflect which induces vibrations in the fluid, the glass capillary and the piezoelectric element itself. These vibrations are in turn converted to a sensor voltage signal 55 by the sensing piezoelectric element 24, 24a. The analog to digital converter (ADC) 56 is then, in step 64, triggered to sample and digitize this signal. The computer then in step 66 stores the digitized sensor signal 57 output values. Steps 64 and 66 are repeated. When the voltage signal is completely sampled, the data is made available for digital signal processing and analysis by the computer. The power spectral densities of Figure 7 are then computed in step 68 using any accepted method of power spectral estimation. The PSD is then evaluated in step 70 by comparing the PSD value at different frequencies to known corresponding values for a particular operational state of the device. For each evaluation, the operational

state of the device 10, 10a is determined in accordance with step 72. Steps 70 and 72 are repeated for all of the various operational states of the device.

Temperature Control

5 Figure 10 shows a piezoelectric pipette 70 wherein the temperature of the glass capillary 72 and thus the fluid 74 within the capillary may be controlled in accordance with a further aspect of the present invention. Here, the actuating element 76 is associated with an outer electrode 78 and an inner electrode 80 having two wrap-around electrode portions 82 and 84 on each end of the
10 piezoelectric crystal 76. Applying a current between the wrap-around electrode portions 82 and 84 and thus through the inner electrode 80 causes the temperature of the device to increase. A thermocouple 86, depicted in Figure 10, is closely adjacent the nozzle 87 and senses the temperature of the device. The temperature may be regulated with a temperature regulator 88 (Figure 11) such as an Analog
15 Devices AD594 as specified in the *Monolithic Thermocouple Amplifier Data Sheet* from Analog Devices. This integrated circuit controls the current passing through the inner electrode to maintain a set-point temperature using feedback from the thermocouple as depicted in the block diagram in Figure 11.

As can be seen in Figure 11, the temperature regulator 88 provides a control
20 output to a heater driver 90 which applies the current to the inner electrode 80. The amount of current applied is dictated by the temperature regulator 88 and more specifically the difference between the output voltage of the thermocouple 86 and a set point reference voltage applied to a subtractor stage 92.

What is claimed is:

1. A diffuser valve pump comprising:
 - a pipette including a capillary having a first end including a nozzle and
 - a piezoelectric actuating element adjacent to the capillary; and
 - 5 an alternating current voltage source coupled across the piezoelectric actuating element, whereby
 - with the nozzle immersed in a sample fluid and the capillary at least partially filled with a working fluid, alternating current voltage applied to the actuating element causes sample fluid to be drawn into the capillary.
- 10 2. A diffuser valve pump as defined in claim 1 wherein the alternating current voltage source provides alternating current voltage having one of a saw-tooth square-wave and sine-wave waveform.
- 15 3. A diffuser valve pump as defined in claim 1 wherein the capillary has a second end opposite the first end and wherein the diffuser valve pump further includes a reservoir attached to the second end of the capillary.
- 20 4. A diffuser valve pump as defined in claim 1 wherein the capillary has a second end opposite the first end and wherein the diffuser valve pump further includes a first conduit attached to the first end and a second conduit attached to the second end whereby, with fluid filling the capillary and the conduits, the valve pump functions as a continuous flow pump.
- 25 5. A diffuser valve pump as defined in claim 1 wherein the capillary includes a second end opposite the first end, wherein the diffuser valve pump further includes a second pipette including a capillary having a first end including a nozzle and a second end opposite the first end and a piezoelectric actuating element adjacent the capillary, wherein the second ends of pipettes are attached for communicating with
- 30 each other, and wherein the diffuser pump valve further includes conduit means attached to the first ends of the pipettes, whereby, with alternating current voltage

selectively applied across the actuating elements, and with fluid filling the capillaries and conduits, the diffuser valve pump is arranged to function as a reversible continuous flow pump.

5 6. A diffuser valve pump as defined in claim 1 further including a mounting arm for disposing the nozzle immersed in a sample fluid.

7. A pipette sensing arrangement for sensing a working condition of a pipette having a capillary having an end including a nozzle, a piezoelectric actuating
10 element adjacent the capillary, and first electrodes for applying electrical energy across the piezoelectric actuating element, the sensing arrangement comprising:
a piezoelectric sensing element adjacent the capillary; and
second electrodes for coupling the piezoelectric sensing element to a
voltage sensor.

15

8. An arrangement as defined in claim 7 wherein the sensing element is proximal to the actuating element with reference to the nozzle.

9. An arrangement as defined in claim 7 wherein the voltage sensor includes
20 an analog to digital converter for generating digital data responsive to the sensing element.

10. An arrangement as defined in claim 9 further including means for analyzing the digital data.

25

11. An arrangement as defined in claim 10 wherein the means for analyzing includes means for generating power spectral densities.

12. An arrangement as defined in claim 11 wherein the means for analyzing
30 further includes comparing means for comparing the generated power spectral

densities at preselected frequencies to predetermined power spectral density values corresponding to the preselected frequencies.

13. A temperature control arrangement for a pipette having a capillary having
5 an end including a nozzle, a piezoelectric actuating element adjacent the capillary, and actuating electrodes for applying electrical energy across the piezoelectric actuating element, the temperature control arrangement comprising:

a current source for passing an electrical current through one of the
actuating electrodes; and

10 a temperature sensor closely adjacent the capillary for sensing the temperature of the capillary.

14. An arrangement as defined in claim 13 wherein the temperature sensor includes a thermocouple.

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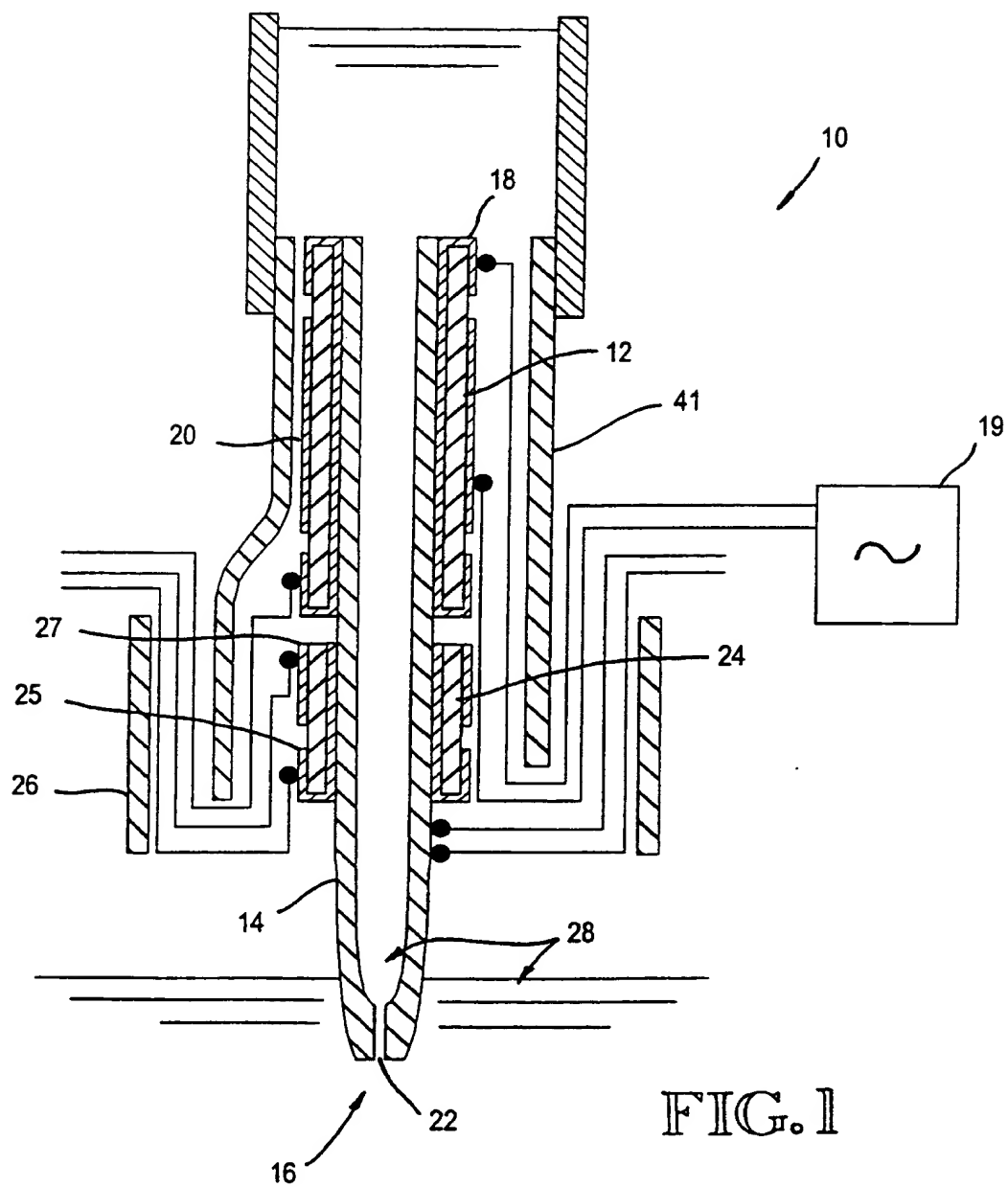
15. An arrangement as defined in claim 13 wherein the temperature sensor is closely adjacent the nozzle.

16. An arrangement as defined in claim 17 further including means for
20 controlling the current source responsive to the temperature sensor.

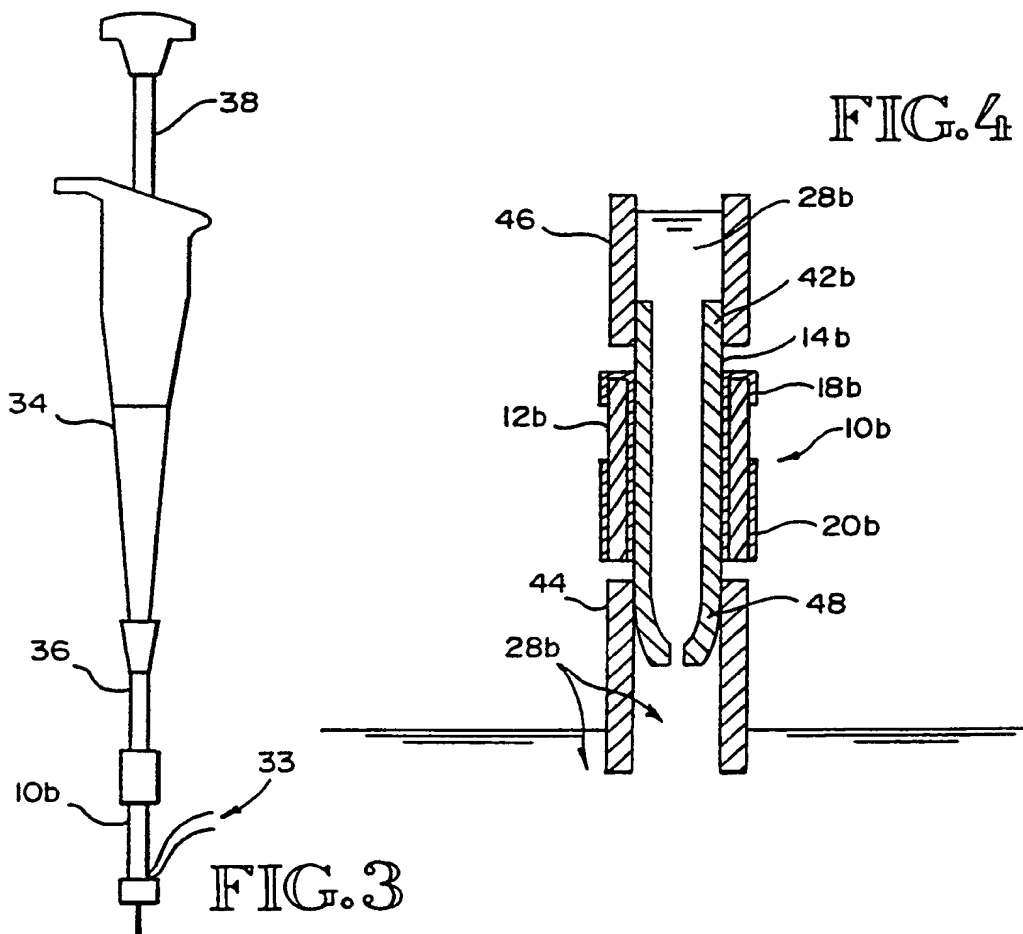
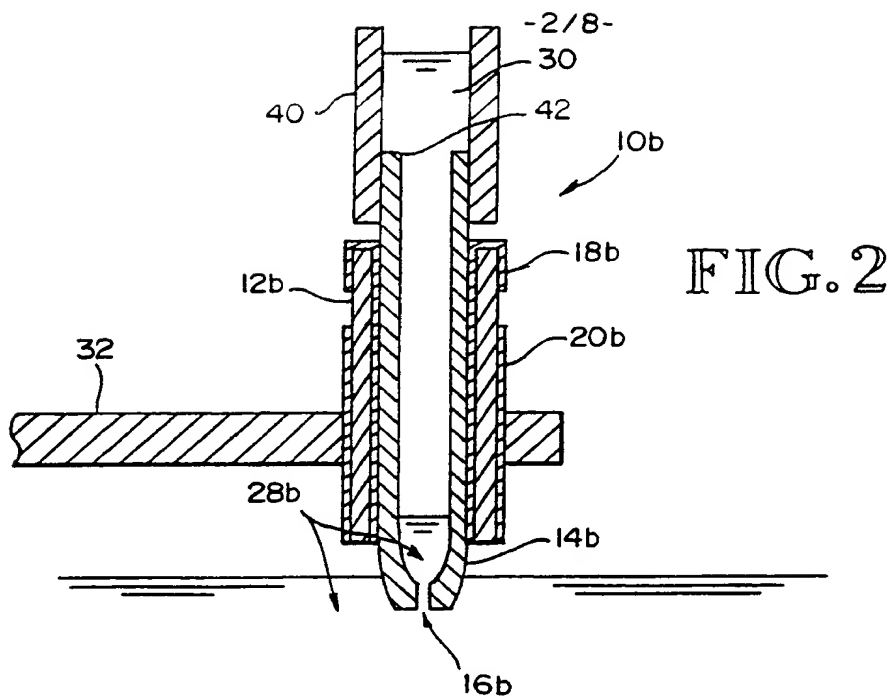
17. An arrangement as defined in claim 16 wherein the temperature sensor includes means for generating a voltage representative of the sensed temperature and wherein the means for controlling is responsive to a difference between the
25 voltage generated by the temperature sensor and a set point reference voltage.

18. An arrangement as defined in claim 13 wherein the actuating electrodes include an inner electrode between the actuating element and the capillary and an outer electrode adjacent the actuating element on a side of the actuating element
30 opposite the capillary, and wherein the current source is coupled to the inner electrode for passing the electrical current through the inner electrode.

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SUBSTITUTE SHEET (RULE 26)



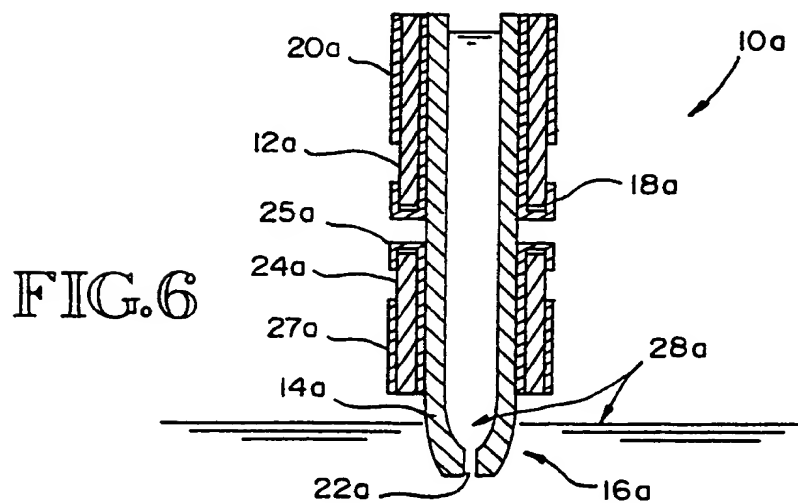
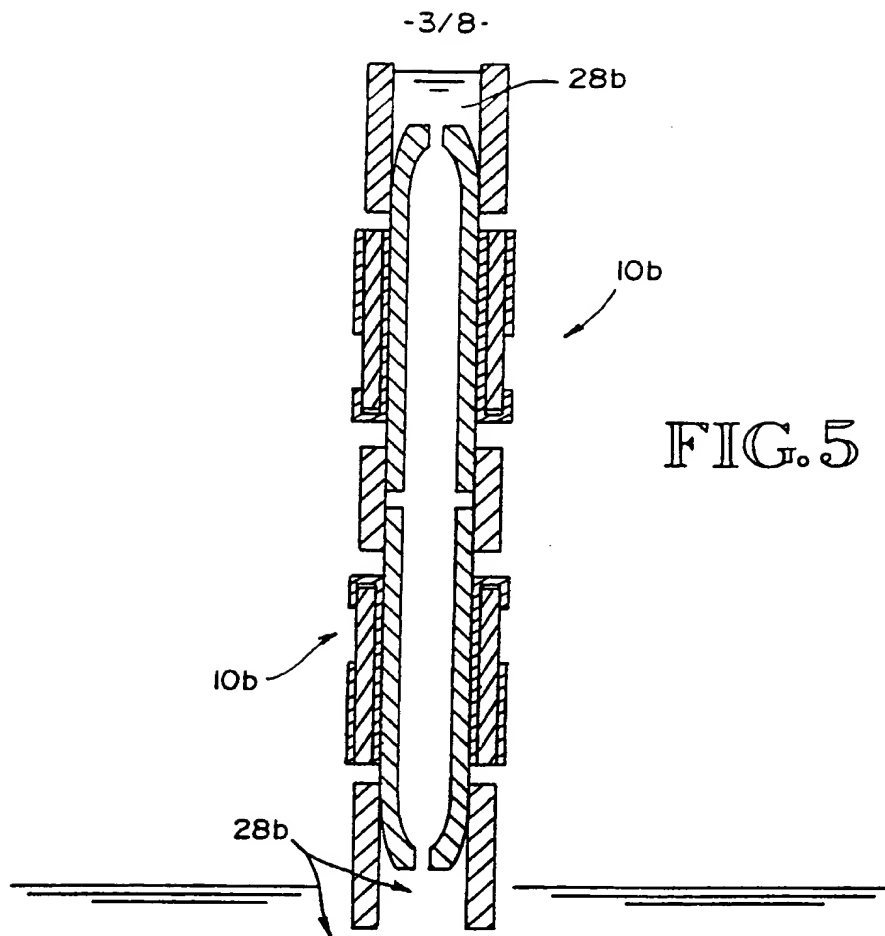
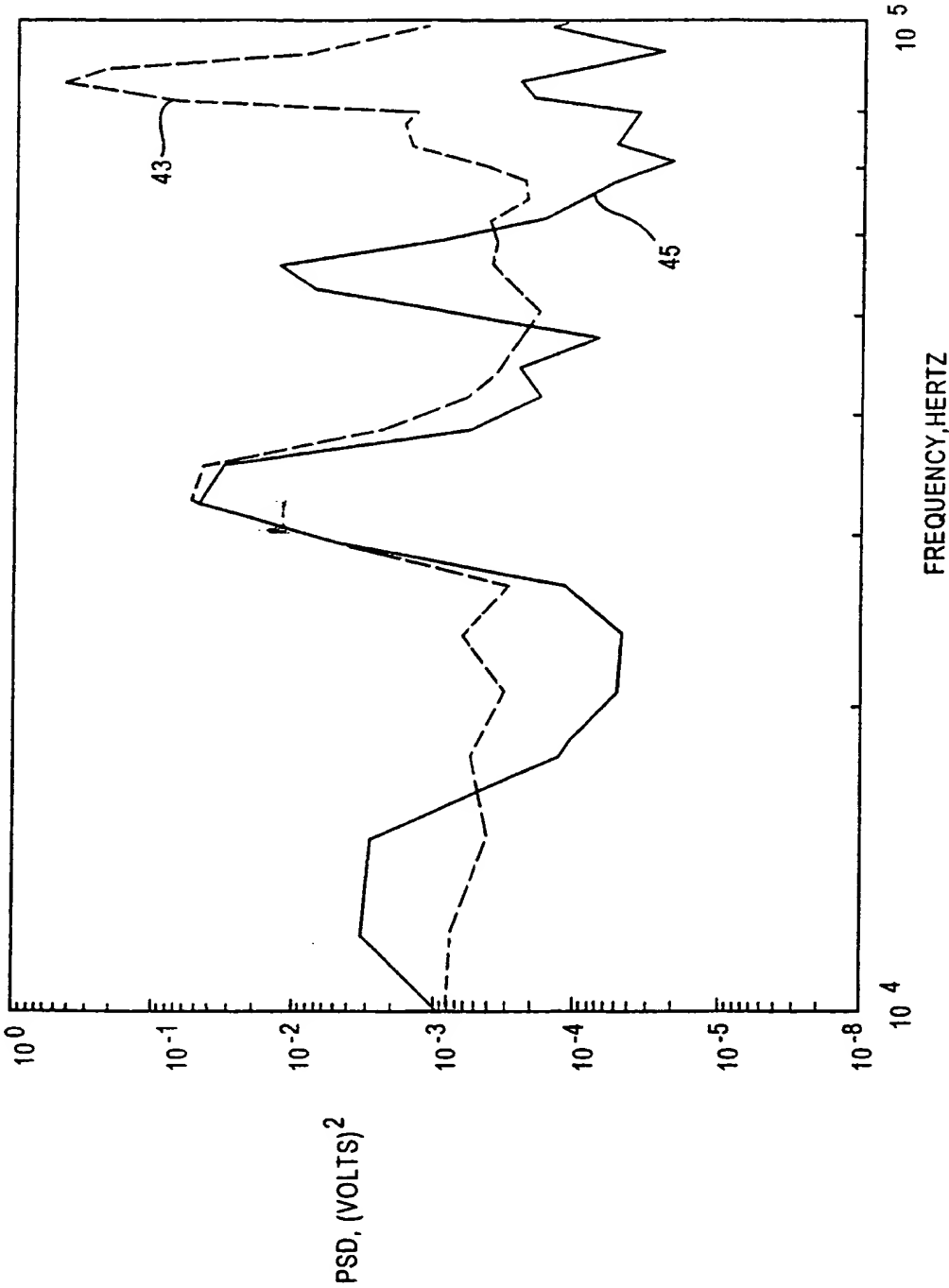


FIG. 7



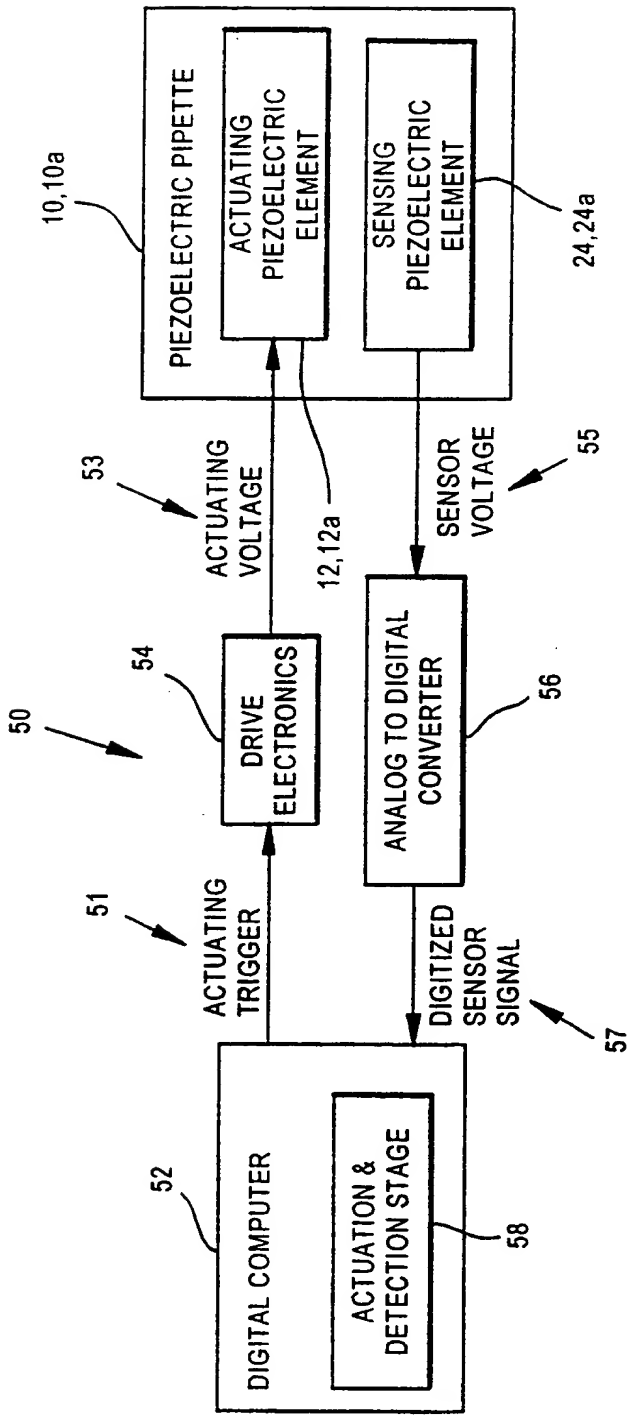
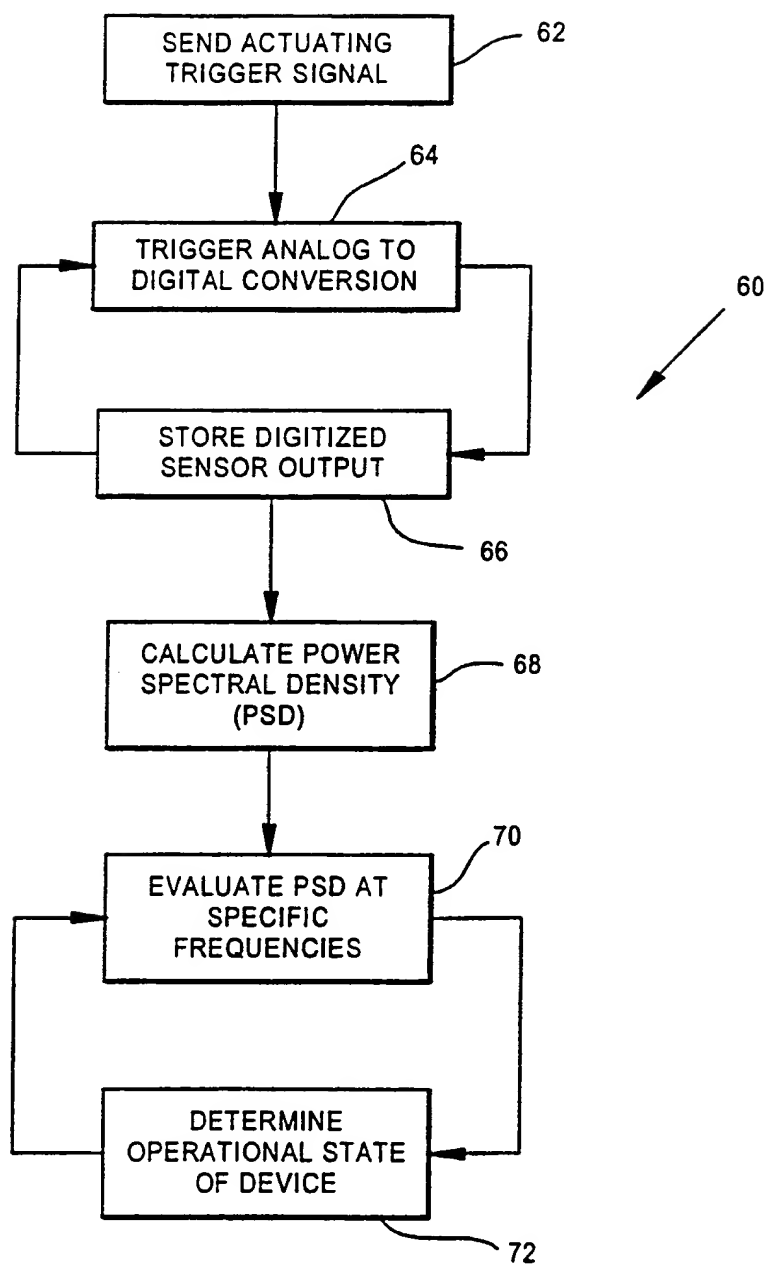


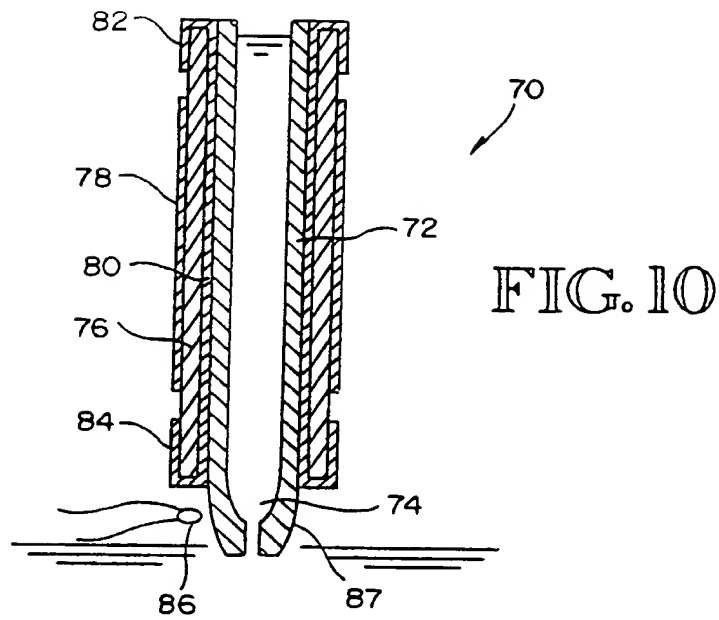
FIG. 8

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FIG. 9

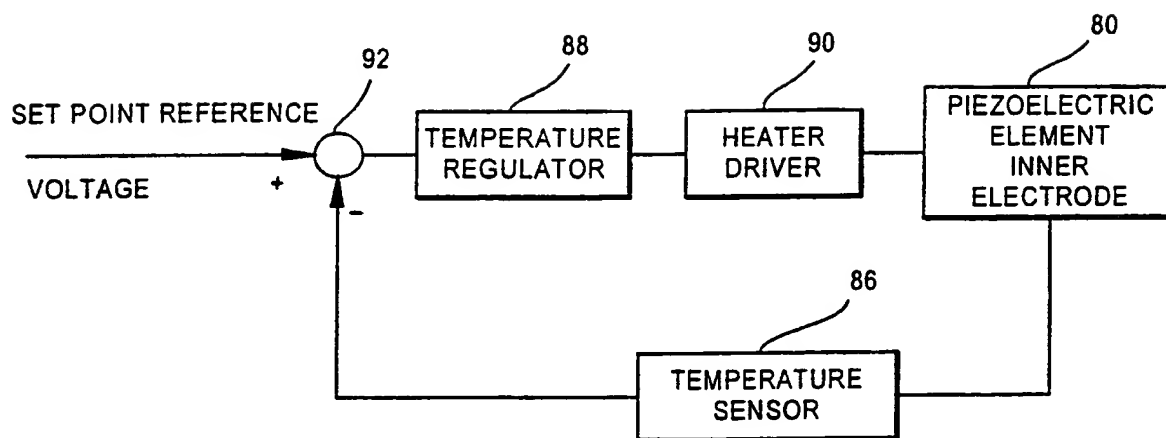


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- 8/8 -

FIG. 11



INTERNATIONAL SEARCH REPORT

Inter. natl Application No

PCT/US 00/02730

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 725 267 A (ROSSENDORF FORSCHZENT) 7 August 1996 (1996-08-07) abstract; figures 1-3 column 3, line 7 -column 5, line 3 column 5, line 49 -column 5, line 55	1-3, 6
A		4, 5, 7, 13
A	US 4 432 699 A (BECKMAN JOHN B ET AL) 21 February 1984 (1984-02-21) cited in the application abstract; figures 1-8 column 1, line 63 -column 2, line 40	4, 5, 7-12
A	WO 98 45205 A (PACKARD INSTRUMENT CO INC) 15 October 1998 (1998-10-15) figures 1, 3 page 7, line 27 -page 8, line 13 page 11, line 22 -page 15, line 7	1, 7, 13
	-/-	

☒ Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search

18 April 2000

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/02730

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 865 824 A (HOFFMANN LA ROCHE) 23 September 1998 (1998-09-23) abstract; figures 1-10 column 4, line 49 -column 7, line 17 column 8, line 9 -column 9, line 54	7,8
A	-----	1,9-13
A	DE 41 02 336 A (BEHRINGWERKE AG) 6 August 1992 (1992-08-06) abstract; figures 1-3 column 3, line 11 -column 4, line 30	13-18
A	----- US 5 178 019 A (KEITER DEAN) 12 January 1993 (1993-01-12) abstract; figures 1,2 -----	13-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/02730

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0725267 A	07-08-1996	AT 173539 T WO 9624040 A DE 59600820 D	15-12-1998 08-08-1996 24-12-1998
US 4432699 A	21-02-1984	US 4449893 A	22-05-1984
WO 9845205 A	15-10-1998	AU 6963798 A EP 0810438 A JP 10114394 A	30-10-1998 03-12-1997 06-05-1998
EP 0865824 A	23-09-1998	JP 10318150 A	02-12-1998
DE 4102336 A	06-08-1992	AU 654995 B AU 1045592 A CA 2058648 A DE 9111441 U JP 6063424 A US 5287758 A	01-12-1994 30-07-1992 27-07-1992 09-01-1992 08-03-1994 22-02-1994
US 5178019 A	12-01-1993	AU 649542 B AU 1762192 A CA 2083486 A EP 0531518 A FI 925341 A PT 100293 A WO 9217761 A ZA 9202108 A	26-05-1994 02-11-1992 27-09-1992 17-03-1993 25-11-1992 29-04-1994 15-10-1992 27-01-1993



P.B.5818 - Patentlaan 2
2280 HV Rijswijk (ZH)
☎ +31 70 340 2040
TX 31651 epo nl
FAX +31 70 340 3016

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Zweigstelle
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recherche

Liesegang, Roland, Dr.-Ing.
FORRESTER & BOEHMERT
Pettenkoferstrasse 20-22
80336 München
ALLEMAGNE

FORRESTER & BOEHMERT

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Anmelder/Applicant/Demandeur/Patentinhaber/Propriétaire/Titulaire

Hitachi Software Engineering Co., Ltd.

COMMUNICATION

The European Patent Office herewith transmits as an enclosure the European search report for the above-mentioned European patent application.

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The following specifications given by the applicant have been approved by the Search Division:

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The following figure will be published together with the abstract: 1

REFUND OF THE SEARCH FEE

If applicable under Article 10 Rules relating to fees, a separate communication from the Receiving Section on the refund of the search fee will be sent later.





DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 98 45205 A (PACKARD INSTRUMENT CO INC) 15 October 1998 (1998-10-15) * abstract * * page 1, line 2 - page 2, line 2 * * page 6, line 15 - line 28 * * page 7, line 27 - page 8, line 12 * * page 23, line 20 - line 23; figure 1 * ---	1-4	B01J19/00
X	US 4 877 745 A (HAYES DONALD J ET AL) 31 October 1989 (1989-10-31) * abstract * * column 1, line 9 - line 12 * * column 2, line 4 - line 9 * * column 18, line 41 - line 51 * ---	1-4	
X	US 5 508 200 A (THAYER PHILLIP ET AL) 16 April 1996 (1996-04-16) * abstract * * column 5, line 40 - column 6, line 17; figures 4,5 * ---	1,2,4	
X	EP 0 947 819 A (HITACHI SOFTWARE ENG) 6 October 1999 (1999-10-06) * abstract * * column 8, line 43 - column 9, line 4 * ---	1,2	TECHNICAL FIELDS SEARCHED (Int.Cl.7) B01J B01L B41J
P,X	WO 00 45955 A (WIKTOR PETER) 10 August 2000 (2000-08-10) * abstract * * page 1, line 4 - line 14 * * page 7, line 5 - line 19 * * page 8, line 8 - line 20; figures 1,2 * -----	1-4	
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 29 April 2003	Examiner Nazario, L
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29-04-2003

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9845205	A	15-10-1998	AU 6963798 A	30-10-1998
			EP 0810438 A2	03-12-1997
			EP 1007973 A2	14-06-2000
			JP 10114394 A	06-05-1998
			US 6203759 B1	20-03-2001
			WO 9845205 A2	15-10-1998
			US 6521187 B1	18-02-2003
			US 6537817 B1	25-03-2003
			US 2001014477 A1	16-08-2001
			US 2001016177 A1	23-08-2001
US 4877745	A	31-10-1989	AT 112849 T	15-10-1994
			AU 603617 B2	22-11-1990
			AU 8120787 A	19-05-1988
			CA 1308467 A1	06-10-1992
			DE 3750655 D1	17-11-1994
			DE 3750655 T2	11-05-1995
			EP 0268237 A2	25-05-1988
			JP 7006975 B	30-01-1995
			JP 63139253 A	11-06-1988
US 5508200	A	16-04-1996	NONE	
EP 0947819	A	06-10-1999	JP 11281534 A	15-10-1999
			EP 0947819 A2	06-10-1999
			US 6127125 A	03-10-2000
WO 0045955	A	10-08-2000	US 6232129 B1	15-05-2001
			CA 2361512 A1	10-08-2000
			EP 1152831 A1	14-11-2001
			WO 0045955 A1	10-08-2000

